Development of SCAR Marker Linked to a Major QTL for High Fiber Strength and Its Usage in Molecular-Marker Assisted Selection in Upland Cotton

Wangzhen Guo, Tianzhen Zhang,* Xinlian Shen, John Z. Yu, and Russell J. Kohel

ABSTRACT

Cotton fiber is a basic raw material in the textile industry. All the changes in spinning technology require unique and often greater cotton fiber quality, especially strength, for processing. On the basis of genetic analysis and molecular mapping, a major quantitative trait locus (QTL) for fiber strength was identified in 7235, an Upland cotton (*Gossypium hirsutum* L.) germplasm line in our institute, which possesses super quality fiber properties. Eight molecular markers, two simple sequence repeat (SSRs) and six random amplified polymorphic DNA (RAPDs) markers, were linked to the QTL. The objective of this study was to develop rapid and reliable sequence characterized amplified region (SCAR) markers linked to the QTL for marker-assisted selection (MAS). Two RAPD markers, $UBC431_{1920}$ and $UBC757_{1365}$, were converted to SCAR markers after sequencing the two ends of the two polymorphic DNA fragments. Only $SCAR431_{1920}$ marker detected polymorphism between TM-1 and 7235, whereas $SCAR757_{1365}$ showed monomorphism. $SCAR431_{1920}$ marker was explored to determine its stability in (7235 $\times$ TM-1) F_2 with $UBC431_{1920}$ marker as a control and to screen the major fiber strength QTL of (7235 $\times$ Simian 3) BC_1F_4 population for transferring good fiber quality. The result showed that the specific $SCAR431_{1920}$ marker could be applied to large-scale screening for the presence or absence of the major fiber strength QTL in breeding populations.

Cotton fiber is an important raw material for the textile industry. As a result of the recent advances in the textile industry in spinning technology, greater cotton fiber quality, especially strength, is required (Deussen, 1992). Because of the rigors of ginning, opening, cleaning, carding, combing, and drafting, an important breeding objective has been to increase fiber strength. Fiber strength in Upland cotton grown in the USA has been increased by 0.19 cN tex^{-1} yr^{-1} over the 10-yr period of 1982 to 1992. The average strength of the commercial cultivars in 1991 to 1992 was close to 21.7cN tex^{-1} (Benedict et al., 1999). There is an urgent need to improve cotton fiber quality further. However, there is no rapid way to do this by current plant breeding methods and on the basis of current genetic information. In traditional breeding to develop super quality fiber properties, the quality of the fiber can be determined only after harvesting and testing the fiber. As a result, it is difficult, expensive, and time-consuming to develop cotton cultivars with super quality fiber by these methods.

Advances in the use of DNA markers for MAS provide a promising method of streamlining plant breeding programs (Kohel et al., 2001; Lawson et al., 1997; Mohan et al., 1997). In 1999-2000, we used a *G. anomalum* Wawra ex Wawra & Peyritch introgression line 7235, which had been determined to possess good fiber quality, to identify molecular markers linked to fiber strength QTLs. By use of F_2 and $F_{2:3}$ populations derived from a cross between 7235 and TM-1, a widely used genetic standard of Upland cottons, a major QTL for fiber strength was identified. Eight molecular markers, two SSRs and six RAPDs, covering 15.6 centimorgans (cM) of genetic distance, were linked to this QTL. It could explain more than 30% of the phenotypic variation. The QTL was detected in different locations, such as Nanjing and Hainan in China, and College Station, TX, in the USA. Therefore, these DNA markers can be used in MAS to increase fiber strength of commercial cultivars (Zhang et al., 2003).

RAPD technology has the shortcoming of relatively low reproducibility and reliability, which limits its use in breeding program, whereas molecular markers based on polymerase chain reaction (PCR) technology should be an efficient means of screening large populations, and they are relatively easy analyze, can be screened at with reproducibility, and are economical. Paran and Michelmore (1993) described a reliable PCR assay that they called sequence characterized amplified regions (SCARs) in which the two ends of the polymorphic DNA fragment are sequenced and two longer primers homologous to each end are then synthesized. Some SCAR markers have been developed for insect and disease resistance and fertility restoration genes (Nair et al., 1995; Nair et al., 1996; Nagvi and Chatto, 1996; Ohmori et al., 1996; Norio, 1997; Liu et al., 1999). These SCAR markers increased reliability and specificity for screening large breeding populations by means of the converted molecular markers for assisting breeding selection. The objective of this study was to develop SCAR markers for molecular identification of the high fiber strength QTL that we identified and to use such markers to select the high fiber strength QTL in cotton breeding programs.

MATERIALS AND METHODS

Plant Materials

Gossypium anomalum introgression germplasm lines were developed by crossing *G. anomalum* with *G. hirsutum*, and then backcrossing to cultivars and strains with high fiber strength, such as Acala 3080, PD 8619, and PD 4381. The germplasm line 7235 was developed from a backcrossed prog-

W. Guo, X. Shen, and T. Zhang, National Key Laboratory of Crop Genetics & Germplasm Enhancement, Cotton Research Inst., Nanjing Agric. Univ., Nanjing 210095, China; J.Z. Yu and R.J. Kohel, USDA ARS, Southern Plain Agriculture Research Center, Crop Germplasm Research Unit, College Station, TX 77845, USA. Received 24 Aug. 2001. *Corresponding author (cotton@njau.edu.cn).

Published in Crop Sci. 43:2252–2256 (2003).

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: MAS, marker-assisted selection; QTL, quantitative trait locus; RAPD, random amplified polymorphic DNA; SCAR, sequence characterized amplified region; SSR, simple sequence repeat.

eny with Acala 3080 (Qian et al., 1992). The fiber strength of 7235 was 27.3 cN tex^{-1}, its fiber length was 35 mm, and its fineness was 4.1 Micronaire units, and it was grown at Nanjing in 1998. TM-1, the genetic standard line for Upland cotton (Kohel et al., 1970), was obtained from USDA ARS, Southern Plains Agriculture Research Center, College Station, TX, USA. Its fiber strength was 20.7 cN tex^{-1}, its length was 30.5 mm, its fiber fineness was 5.0 Micronaire units, and it was grown at Nanjing in 1998.

The same (7235 × TM-1) F_2 population that was used for the tagging QTLs for fiber qualities (Zhang et al., 2003) was also used to identify the consistency of the objective marker after using $UBC431_{1920}$ and $SCAR431_{1920}$ to screen separately. To transfer the superior fiber quality gene from 7235 to commercial cultivars, Simian 3 (SM 3) in Yantze River valley was crossed with 7235. From (7235 × SM 3) BC_1F_1 to (7235 × SM 3) BC_1F_4 generations, individual plants were selected on the basis of fiber quality detection results. Individual plants selected from (7235 × SM 3) BC_1F_4 population were planted into families at the Jiangpu Breeding Station and Nanjing Agricultural University in 2000. Individual plants that were used to test the genetic stability of the QTL and its MAS result ranged from 5 to 8 in each family. All the cotton fiber from each individual plant from (7235 × SM 3) BC_1F_4 was harvested for the fiber tests. Fiber samples were tested by Supervision, Inspection & Test Center of Cotton Quality, the Ministry of Agriculture, China. The difference in the fiber quality of the individual plants with and without the molecular marker in this advanced backcrossing/selfing population was determined by the *t* test.

DNA Analysis

Total DNA was extracted from a healthy leaf of each of the (TM-1 × 7235) F_2 plants in the breeding population by the cetyltrimethylammonium bromide (CTAB) method (Paterson et al., 1993) with minor modifications in the elimination of diethyldithiocarbamic acid. Following a single chloroform-isoamyl alcohol (24:1) extraction, the precipitated DNA was resuspended in sterile TE for amplification by PCR.

All PCR amplifications were performed in 20-μL volumes in a Perkin-Elmer 9600 Thermocycler (Foster City, CA). RAPD experiments included 45 cycles of amplification (at 95°C for 30 s, at 40°C for 1 min, at 72°C for 1.5 min after an initial denaturing step at 95°C for 2 min), performed in a 25-μL reaction volume containing 0.5 units *Taq* DNA polymerase (Sigma, St. Louis, MO), a buffer (100 m*M* Tris-HCl, pH 8.3, 500 m*M* KCl), a 0.5 μ*M* primer, 100 μ*M* dNTP (dATP, dTTP, dGTP, dCTP), and 3.5 m*M* of $MgCl_2$ supplied by the enzyme manufacturer. PCR products were separated in 1.4% (w/v) agarose gels and visualized under UV light after ethidium bromide staining. SSR reactions contained 67 m*M*/L of Tris-HCL (pH 8.8), 16 m*M*/L of $(NH_4)_2SO_4$, 0.002% (w/v) gelatin, 0.2 m*M*/L dNTPs, 2.5 m*M*/L of $MgCl_2$, F-primer and R-primer (Research Genetics, Huntsville, AL) 0.6μ*M*/L separately, 0.25U of Taq DNA polymerase (Sangon Company, Shanghai) and a template DNA of 20 ng. The amplification was programmed at 95°C for 2 min, followed by 30 cycles at 94°C for 40 s, then at 57°C for 45 s and then at 72°C for 1min, with a final extension at 72°C for 7 min. PCR products were inspected by means of polyacrylamide gel electrophoresis–silver staining. SCAR reactions contained 10 mM of Tris-HCl (pH 8.3), 50 m*M* of KCl, 2.5 m*M* of $MgCl_2$, 200 μ*M* of each dNTP, 1 μ*M* of each pair of primers, 20 ng of total DNA, and 1 U of Taq DNA polymerase. The amplification was programmed at 95°C for 2 min, followed by 35 cycles at 94°C for 30 s, then at 58°C for 1min, then at 72°C for 1min 30 s, with a final extension at 72°C for 10 min. PCR products were separated on a 1.0% (w/v) agarose gel.

The 1365- and 1920-basepair (bp) polymorphic RAPD fragments, amplified from 7235 by means of random primer UBC757 (GGAAGGGAGG) and UBC431 (CTGCGGG TCA), were cut from the agarose gel with a clean scalpel and the agarose plug containing the DNA was placed in a 1.5-mL microcentrifuge tube with 100 μL of TE. The samples were heated at 94°C for 15 min to dissolve the agarose, then diluted with TE solution according to different proportion, such as 1:25,1:50, and 1:100 separately, and 1-μL aliquots of each dilution under the same condition described before with the same primers were reamplified. The amplification products were directly ligated to pGEM-T easy vector (Promega, Madison, WI, USA) after they were confirmed to be RAPD bands through running gel on 2% (w/v) agarose for each dilution as recommended by the manufacturer. The recombinant vectors were separately used to transform competent *Escherichia coli* DH5α cells. The different clones, p1365 and p1920, were sequenced on an ABI Prism 377 DNA Sequencer (PE Biosystems, Foster City, CA) after the correct insert size was confirmed by *Eco*RI digestion. Double-strand sequencing was done by means of the dideoxy-chain termination method with SP6 and T7 universal primers. The sequencing experiment was conducted by Shanghai Sangon Company.

RESULTS

Cloning and Sequencing of $UBC431_{1920}$ and $UBC\ 757_{1365}$

To develop a reliable SCAR marker for the detection of $UBC431_{1920}$ and $UBC757_{1365,}$ the polymorphic DNA fragments, with 1920- and 1365-bp molecular weights amplified from 7235, were cloned separately into the pGEM-T easy vector. When the inserted DNA from different clones was digested with *Eco*RI (Fig. 1), it was found that the 1920-bp fragment from UBC431 had an *Eco*RI restriction site at about 1000 bp from the 5' end of the sequences and that the 1365-bp fragment from UBC757 had no such restriction site. The two different clones, p431-1920 and p757-1365, were sequenced after the correct insert size was confirmed by *Eco*RI digestion. The sequencing results confirmed that both ends of the cloned fragments were consistent with the 10-base random primers of UBC431 and UBC757.

Development of SCAR Markers Linked to High Fiber Strength QTLs

On the basis of the sequences of the two ends in p431-1920 and p757-1365, two pairs of 21 to 24 bases long (P1F and P1R, P2F and P2R), which included the original 10 bases of random primer of UBC431 and UBC757, respectively, were designed.

p431-1920F: CTGCGGGTCATGATCTGAAAT
p431-1920R: CTGCGGGTCAAGGATATTATCAG
p757-1365F: GGAAGGGAGGCGTCAATAAAGT
p757-1365R: GGAAGGGAGGGAAAACTATTGG

Primer combination p757-1365F and p757-1365R amplified a 1365-bp DNA fragment not only in high strength parent 7235 and its $F_{1,}$ but also in TM-1, which suggested that the pair of primers could not be used in

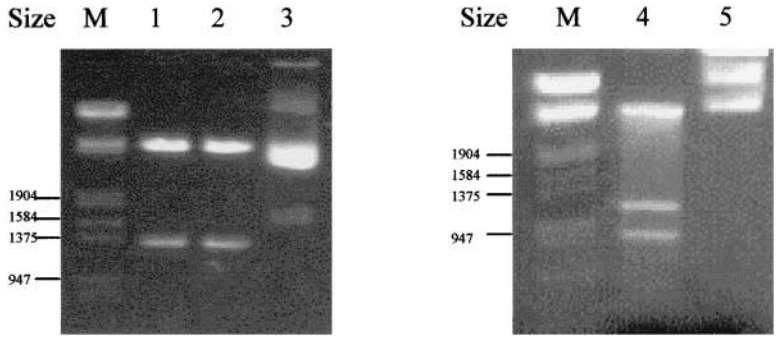

Fig. 1. *Eco*RI digestion of the recombinant clones. Lane 1-2: *Eco*RI digested p757-1365; Lane 3: undigested p757-1365; Lane 4: *Eco*RI digested p431-1920; Lane 5: undigested p431-1920; and M: Lambda DNA/*Eco*RI + *Hin*dIII.

assisted selection for high strength fiber QTLs. Primer combination p431-1920F and p431-1920R (designated as SCAR431) amplified a 1920-bp DNA fragment only in the donor parent 7235 and its F_1, but not in TM-1 (Fig. 2). To identify the consistency of amplification results by primers UBC431 and SCAR431, further investigation was conducted with the same (7235×TM-1) F_2 segregation population as was used in the tagging of high strength fiber QTLs (Zhang et al., 2003). In a total of 90 RAPD reactions, 17 (18.9%) yielded no $UBC431_{1920}$ products, while 73 (81.1%) had the expected result. SCAR431 primer combinations also amplified the same 90 individuals of the (7235×TM-1) F_2 population; the results of producing targeted polymorphic bands were nearly 96% consistence with those that used UBC431 primer. The difference is that the products amplified with UBC431 primer were weak, unstable, and difficult to identify (Table 1).

Evaluation of the RAPD, SSR, and SCAR Markers Linked to High Strength Fiber QTLs for Selecting High Strength Plants

On the basis of our having crossed (SM 3×7235) BC_1F_4 families for transferring high fiber strength QTLs, we selected five to eight individual plants from each family to test the advantage of the SCAR marker over the RAPD markers in the detection of high strength fiber QTLs. This was done to determine the genetic stability of the QTL and its MAS. A total of 164 high strength single plants from (SM 3×7235) BC_1F_4 derivatives were tested for fiber quality by means of $SCAR431_{1920}$ and were subjected to the analysis of two

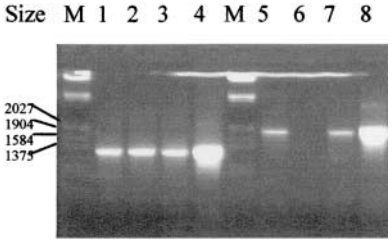

Fig. 2. Development of SCAR markers for $UBC757_{1365}$ and $UBC431_{1920}$. Lanes 1, 2, 3, and 4 show products of 7235, TM-1, F_1 and p757-1365 amplified with a pair of primer, P2F and P2R; lanes 5,6,7, and 8 show products of 7235, TM-1, F_1 and p431-1920 amplified with a pair of primer, P1F and P1R; and M, Lambda DNA/*Eco*RI + *Hin*dIII.

RAPD markers ($UBC301_{933}$ and $OPM07_{1047}$) and one SSR marker ($SSR1521_{130}$) as a control. $SCAR431_{1920}$ produced clearly targeted products in the breeding population (Fig. 3). Compared with the result derived from screening with two RAPD primers and one SSR marker (Table 2), the consistency rates, in terms of presence or absence of the targeted product, were 88.4% for $UBC301_{933}$ and $OPM07_{1047}$, 92.1% for $UBC301_{933}$ and $SCAR431_{1920}$, 96.3% for $SCAR431_{1920}$ and $OPM07_{1047}$, and 92.1% for $SCAR431_{1920}$ and $SSR1521_{130}$, respectively. Some crossovers occurred among these three markers. The genetic distance was 6.7 cM between $UBC431_{1920}$ and $OPM07_{1047}$, 4.0 cM between $UBC431_{1920}$ and $SSR1521_{130}$, and 8.9 cM between $UBC431_{1920}$ and $UBC301_{933}$, the pair of markers with the most frequent crossovers. The result was consistent with the linkage map shown in Fig. 4. All the individuals having the marker showed high fiber strength. Fiber strength of SM 3, a commercial cultivar, was 22.00cN tex^{-1} in 2001.

DISCUSSION

Fiber strength is typically a quantitatively inherited trait (see review by May, 1999). Additive gene action predominates and five (Self and Henderson 1954) to as many as 14 (Tipton et al., 1964) genes were found to influence fiber strength. However, some data suggested that fiber strength might not always segregate in a quantitative manner, the inheritance analysis of some introgression lines particularly suggested this. Richmond (1951) indicated that recovery of high strength segregants from small backcross population during introgression of the triple hybrid *G. thurberi* Todaro × *G. arboreum* L. × *G. hirsutum* L. was evidence for only a few major genes controlling fiber strength. Meredith (1977, 1992) came to a similar conclusion. Two further backcrosses were made to Deltapline 16 nectareless to produce a nectariless strain, MD65-11, which had strength about 10% higher than that of Deltapine 16. MD 65-

Table 1. Comparison of $UBC431_{1920}$ and $SCAR431_{1920}$ markers for the detection of the high strength QTL in (7235×TM-1) F_2 population.

Markers	Plants for PCR amplification	Number of amplified plants	Number of nonamplified plants
$UBC431_{1920}$	90	73 (81.1%)	17 (18.9%)
$SCAR431_{1920}$	90	76 (84.4%)	14 (15.5%)

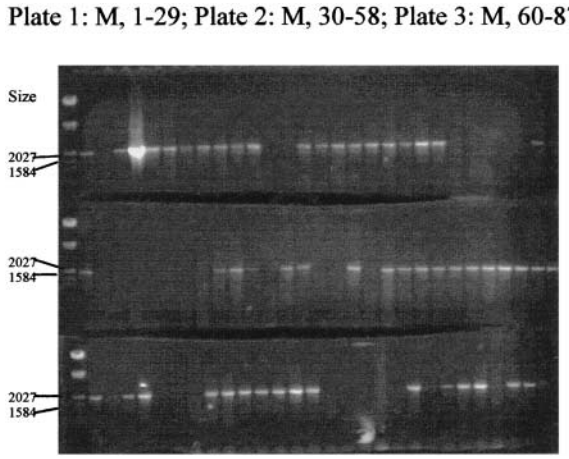

Fig. 3. The amplified results with SCAR431$_{1920}$ in backcrossing/selfing breeding population M, Lambda DNA/*Eco*RI + *Hin*dIII. Lane 1-4 showed amplification product of 7235, TM-1, F_1 and p431-1920; and lanes 5-87 represented different high strength individual plant from (SM 3×7235) BC_1F_4 families.

11 was crossed with Deltapine 90 followed by two backcrosses to Deltapine 90 with selection first for nectareless and then for bundle strength. Genetic analysis of variance components suggested that the 9% increase in strength could be conditioned by no more than a single gene. However, subsequent analysis with larger population sizes indicated that two major genes, which might be linked, were responsible for the high strength trait. From our genetic and molecular tagging results, we concluded that the higher fiber strength trait in 7235 was controlled by one major gene and a polygene (Yuan, 2000). Thus, it is feasible that selection by means of the high strength major QTL marker from 7235 will be successful.

Molecular markers are useful in plant breeding because the presence of targeted traits can be detected without waiting for the phenotypic expression of the gene (Ribaut et al., 1997). Furthermore, molecular markers can be used simultaneously to improve lint yield and fiber strength, which has become a major breeding consideration owing to a strong negative association between lint yield and fiber strength. In this study, SCAR431$_{1920}$ could be used for selecting high strength individual in the segregating population. The genetic stability of a major QTL for fiber strength and its efficiency of MAS were studied in 164 progenies in BC_1F_4 generations of SM 3×7235 with 7235 as a parent with two RAPD markers, UBC301$_{933}$ and OPM07$_{1047}$, and a SSR marker, with SSR1521$_{130}$ as a control. The

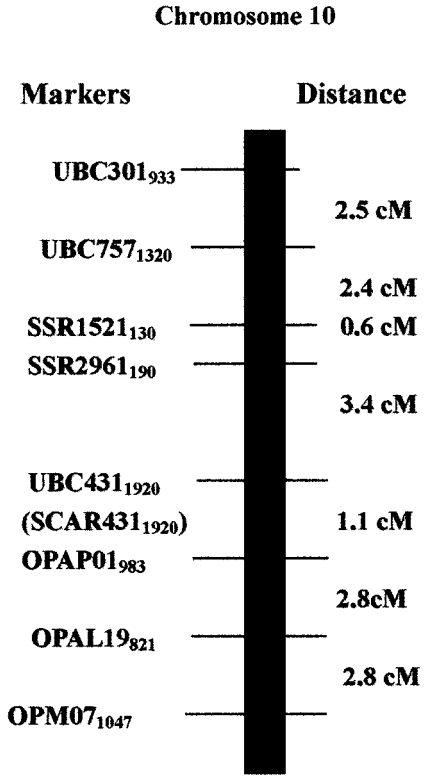

Fig. 4. A chromosome 10 segment showing molecular markers linked to the major QTL for fiber strength in Upland cotton 7235.

results revealed that the QTL for fiber strength associated with the four inherited markers steadily advanced in segregating generation populations. The mean difference of fiber strength between individuals with or without these markers did not decrease after several generations of selfing and backcrossing. There was a significant difference, 25.98 and 24.04 cN tex^{-1}, 25.68 and 24.03 cN tex^{-1}, and 26.14 and 23.66 cN tex^{-1}, in the mean of fiber strength with and without SCAR431$_{1920}$, UBC301$_{933}$, and OPM07$_{1047}$, respectively. Additionally, the fiber strength means of homozygote plants with and without SSR1521$_{130}$ marker were 25.94 cN tex^{-1} and 24.03 cN tex^{-1}. Their difference was significant too. The mean fiber strength of heterozygote identified by SSR marker SSR1521$_{130}$ was 24.94 cN tex^{-1}. The difference between the heterozygote and other genotypes was not significant (Shen et al., 2001). It was concluded that MAS done to increase fiber strength is only possible with this major QTL, but a SCAR marker that is highly reproducible and economical makes it possible to screen

Table 2. Performance of fiber strength with and without DNA markers in (SM 3×7235) BC_1F_4 plants in 2000.

	UBC301$_{933}$		OPM07$_{1047}$		SCAR431$_{1920}$		SSR1521$_{130}$		
Characters	**MPT†**	**WMPT**	**MPT**	**WMPT**	**MPT**	**WMPT**	**HH‡**	**Hh**	**hh**
Number of plants amplified	129	35	110	54	116	48	95	34	35
Fiber strength cN/tex	25.68	24.03	26.14	23.66	25.98	24.04	25.94	24.94	24.03
Difference	1.65		2.48		1.94		1.00	0.91	
Variation	7.74	2.48	6.66	3.56	6.86	2.83	7.49	7.37	2.64
***t* Test**	4.48		6.97		5.36		1.85	1.67	
Probability	2.1×10^{-5}		1.21×10^{-10}		2.3×10^{-10}		0.07	0.19	

† MPT means marked plant type and WMPT without marked plant type.
‡ HH: homozygous for SSR marker, Hh: heterozygous, and hh: without SSR marker.

large breeding populations. Furthermore, a SCAR markers based on PCR technology facilitate molecular analysis of marker-assisted breeding selection.

ACKNOWLEDGMENTS

This project was financially supported in part by National Natural Science Foundation in China (30270806, 30070483), the 863 project in China (2001AA211101), the National Transgenic Program in China (J99-A-023), and The Natural Science Foundation of Jiangsu Province (BK99052).

REFERENCES

Benedict, C.R., R.J. Kohel, and H.L. Lewis. 1999. Cotton fiber quality. p. 269–288. *In* WC Smith (ed.) Cotton: Origin, history, technology, and production. John Wiley & Sons, New York.

Deussen, H. 1992. Improved cotton fiber properties: The textile industry's key to success in global competition. p. 43–64. *In* Proceedings from Cotton Fiber Cellulose: Structure, Function and Utilization Conference. National Cotton Council of America, Memphis, TN.

Kohel, R.J., T.R. Richmond, and C.F. Lewis. 1970. Texas marker-1. Description of a genetic standard for *Gossypium hirsutum* L. Crop Sci. 10:670–671.

Kohel, R.J., J. Yu, Y.-H. Park, and G.R. Lazo 2001. Molecular mapping and characterization of genes controlling fiber quality in cotton. Euphytica 121:163–172.

Lawson, D.M., C.F. Lande, and M.A. Mutscher. 1997. Marker-assisted transfer of acylsugar-mediated Pest resistance from the wild tomato *Lycopersicon pennelli* to the cultivated tomato, *Lycopersicon esculentum*. Mol. Breed. 3:307–317.

Liu, Z., Q. Sun, Z. Ni, and T. Tang. 1999. Development of SCAR markers linked to the *Pm21* gene conferring resistance to powdery mildew in common wheat. Plant Breed. 118:215–219.

May, O.L. 1999. Genetic variation in fiber quality. p.183–230. *In* A.S. Basra (ed.) Cotton fibers, developmental biology, quality improvement and textile processing. Food Products Press, New York.

Meredith, W.R.J. 1977. Backcross breeding to increase fiber strength of cotton. Crop Sci. 17:172–175.

Meredith, W.R.J. 1992 Improving fiber strength through genetics and breeding. p. 289–302. *In* Proceedings from Cotton Fiber Cellulose: Structure, Function and Utilization Conference. National Cotton Council of America, Memphis, TN.

Mohan, M., S. Nair, A. Bhagwat, T.G. Krishna, M. Yano, C.R. Bhatia, and T. Sasaki. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed. 3:87–103.

Nagvi, N.I., and B.B. Chatto. 1996. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome 39:26–30.

Nair, S., J.S. Bentur, U.P. Rao, and M. Mohan. 1995. DNA markers tightly linked to a gall midge resistance gene (*Gm2*) are potentially useful for marker-aided selection in rice breeding. Theor. Appl. Genet. 91:68–73.

Nair, S., A. Kumar, M.N. Srivastava, and M. Mohan. 1996. PCR-based DNA Markers linked to a gall midge resistance gene, *Gm4t*, has potential for marker-aided selection in rice. Theor. Appl. Genet. 92:660–665.

Norio, I. 1997. A rapid PCR-aided selection of a rice line containing the–Rf-1 gene which is involved in restoration of the cytoplasmic male sterility. Mol. Breed. 3:195–202.

Ohmori, T., M. Murata, and F. Motoyoshi. 1996. Molecular characterization of RAPD and SCAR markers linked to the Tm-1 locus in tomato. Theor. Appl. Genet. 92:151–156.

Paran, I., and R.W. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85:985–993.

Paterson, A.H., L. Brubaker, and J. Wendel. 1993. A rapid method for extraction of cotton genomic DNA suitable for RFLP or PCR analysis. Plant Mol. Biol. Rep. 11:122–127.

Qian, S.Y., J.Q Huang., Y.J. Peng, B.L. Zhou, M.C. Ying, D.Z. Shen, G.L.Liu, T.X. Hu, Y.J. Xu, L.M. Gu, W.C. Ni, and S. Chen. 1992. Studies on the hybrid of G.hirsutum L.and G.anomalum Wawr. & Peyr. and application in breeding (in Chinese with English abstract). Scientia Agricultura Sinica 25:44–51.

Ribaut, J.M., X. Hu, and D.A. Hoisington. 1997. Use of STSs and SSRs as rapid and reliable pre-selection tools in a marker-assisted selection–backcross scheme. Plant Mol. Bio. Rep. 15:154–162.

Richmond, T.R. 1951. Procedures and methods of cotton breeding with special reference to American cultivated species. Adv. Genet. 4:213–245.

Self, F.W., and M.T. Henderson. 1954. Inheritance of fiber strength in a cross between the upland cotton varieties AHA50 and Half. Agron. J. 46:151–154.

Shen, X.L., Y.L. Yuan, W.Z. Guo, X.F. Zhu, and T.Z. Zhang. 2001. Genetic stability of a major QTL for fiber strength and its marker-assisted selection in upland cotton. High Tech. Lett. 11:13–21.

Tipton, K.W., M.A.A. El-Sharkawy, B.M. Thomas, J.E. Jones, and M.T. Henderson. 1964. Inheritance of fiber strength in two separate crosses of Upland cotton having a common parent. p. 20–27. *In* J.B.Pate (ed.) Proceedings of the Sixteenth Annual Cotton Improvement Conferences. National Cotton Council of America, Memphis, TN.

Yuan, Y.L. 2000. Inheritance of super fiber quality characters and their molecular tagging in upland cotton (*G. hirsutum* L.). Ph.D. Diss. Nanjing Agric. Univ., Nanjing, China.

Zhang, T.Z., Y.L. Yuan, J. Yu, W.Z. Guo, and R.J. Kohel. 2003. Molecular tagging of a major QTL for fiber strength in upland cotton and its marker-assisted selection. Theor. Appl. Genet. 106:262–268.